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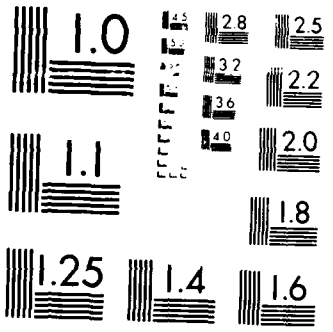

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(U) Molecular Specificity of Adsorption of Biofilm Macromolecules and Microbial Biofouling on Artificial Surfaces in the Sea

12 PERSONAL AUTHOR(S)
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19. ABSTRACT (Continue on reverse if necessary and identify by block number)

The diversity and variability of macromolecular components that mediate initial microbial attachment to surfaces in ambient seawater is beieng determined employing immunological techniques. Antisera against microbial adhesion enhancing (MAE) macromolecules have been raised in chickens and the immunoglobin-G (IgG) fraction utilized for isolation of crude MAE macromolecules from samples of cell free coastal seawater and marine microbial culture media by immunoaffinity chromatography. The objective of the present study is to produce monoclonal antibodies (MAbs) against AE macromolecules, and use these to chromatographically isolate and purify individual MAE macromolecules from mixtures of such components. Variability and diversity of MAE macromolecules produced by biofouling microorganisms, those found in ambient seawater and on biofouled surfaces will be assessed employing a spectrum of AE MAbs. The specificity of the interactions of the various MAE macromolecules with glass and metallic surfaces will be assessed. Alterations in the physical characteristics of the

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DATE 23 June 1988

PROGRESS REPORT ON CONTRACT N00014-88-K-0131

TITLE: The Molecular Specificity of Adsorption of Biofilm Macromolecules and Accumulation of Microbial Biofouling on Artificial Surfaces in the Sea.

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RESEARCH OBJECTIVES:

The diversity and variability of macromolecular components that mediate initial microbial attachment to surfaces in ambient seawater is being determined employing immunological techniques. Antisera against microbial adhesion enhancing macromolecules (MAEM) have been raised in chickens and the immunoglobin-G (IgG) fraction utilized for isolation of crude MAEM from samples of cell free coastal seawater and marine microbial culture media by immunoaffinity chromatography. The objective of the present study is to produce monoclonal antibodies (MAbs) against MAEM and use these to chromatographically isolate and purify individual MAEM from mixtures of such components. Variability and diversity of MAEM produced by biofouling microorganisms, those found soluble in ambient seawater and on biofouled surfaces will be assessed employing a spectrum of MAEM MAbs. The specificity of the interactions of the various MAEM with glass and metallic surfaces will be assessed. Alterations in the physical characteristics of the test surfaces and changes in their susceptibility to microbial biofouling will be correlated with these interactions.

PROGRESS TO DATE:

1. Preparation of Adhesion Enhancing Macromolecular Antigens.

Microbial adhesion enhancing macromolecules (MAEM) have been isolated by immunoaffinity chromatography from samples of cell free coastal seawater and from the laboratory culture growth media of marine microorganisms recovered from biofouled surfaces. MAEM were recovered from these samples by immunoaffinity chromatography employing polyclonal antibodies against these macromolecules. A total of 25.16 mg of MAEM antigens, and 38.08 mg of crude high molecular weight components isolated from the samples cited above over the past 5 years were individually pooled for use in the production of monoclonal antibodies (MAbs).

2. Immunization of Mice with MAEM Antigens.

In the present effort, mice were hyper-immunized with MAEM in an effort to produce high levels of antibody production, in order to optimize subsequent hybridoma production. Initially, groups of mice (10) were given weekly sub-cutaneous (s.c.) injections of 100 micro gm quantities of MAEM (polyclonal antigens described above) for a period of 7 weeks. Serum collected from these animals showed no significant antibody production when tested by indirect ELIZA for anti-MAEM antibodies. MAEM antigen dosages were increased to 500 micro gm/animal in an effort to stimulate antibody production. These analyses are now in progress.

Splenic lymphocytes obtained from mice after single injections of antigen have been successfully employed to produce hybridomas in the case of a number of antigens other than MAEM. Therefore, mice were immunized by single s.c. injections of MAEM, sacrificed 4 days later and their spleens removed for hybridoma production.

3. Hybridoma Production.

Isolated spleens from immunized mice (single s.c. dose) were teased apart in sterile media (HBSS), suspended cells washed and harvested on Ficoll-Hypague density gradients by centrifugation (450 x g, 20'). Hybridization of isolated lymphocytes with murine myeloma cells (SP 2/0) was done by a standard polyethylene glycol (PEG) fusion method. Resultant cell preparations were cultured for 3 weeks in 96 well, flat bottom plates (3), using HAT-supplemented alpha-modified MEM (GIBCO), without nucleosides. At the end of this period, culture supernatants of each well were tested for mouse antibody (IgG or IgM) production by indirect ELIZA. To date 335 colonies have been tested for immunoglobin production, of which 50% were found to be producers (sensitivity 1-3 ng/ml). None of the culture supernatants of these immunoglobin producers tested to date (65) by this method have showed the presence of MAbs that specifically bind MAEM.

WORKPLAN (Remainder of Year 1):

Increased dosages of MAEM antigens (500 micro gm/injection) will be employed in both the hyperimmunization as well as the single s.c dose immunization procedures. No positive anti-MAEM mouse serum is available to standardize the ELIZA assay. In order to ascertain if the ELIZA system is not detecting anti-MAEM antibodies, pooled serum samples will also be tested by double immunodiffusion on agarose slides. All sera tested to date have been negative when tested by this method.

Hybridoma colony production rates were poor initially, during the period in which technicial personnel were being trained, however, with increasing expertise, colony production has risen to 250-270 per spleen, close to the rate (300 colonies/spleen) we wish to achieve. Major effort will be expended to facilitate significant anti-MAEM antibody production in the mice.

WORKPLAN (Year 2):

Assess variability and diversity of MAEM produced by biofouling microorganisms in laboratory culture and those found soluble in samples of ambient coastal seawater. MAEM will be isolated using monoclonal antibodies. Variability, diversity and adhesion enhancement activity of MAEM will be defined immunologically, chromatographically, electrophoretically, and by enumeration of surface adhered microbes using fluorescent microscopy. Define specifity of interactions between MAEM and artificial surfaces. Interaction of MAEM with artificial test surfaces will be assessed using labeled antibodies and microbial adhesion assays, to determine the time dependent minimal accumulation of MAEM required for competent interaction of such surfaces with biofouling microorganisms. Employing these and similar techniques, the persistence and turnover times of surface adsorbed MAEM will be ascertained. These interactions will be correlated with effects of adsorbed MAEM on physical characteristics (free energy, charge and streaming potential) of test artificial surfaces.

INVENTIONS:

None

PUBLICATIONS AND PRESENTATIONS:

None

TRAINING ACTIVITIES:

None

AWARDS AND FELLOWSHIPS:

None

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